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TITLE: Integrated Genomic Biomarkers to Identify Aggressive Disease in African Americans with Prostate Cancer

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13. SUPPLEMENTARY NOTES

14. ABSTRACT
The purpose of our research is to identify somatic copy number alterations and methylation markers in the primary tumors of African American (AA) men that can serve as a component of their recurrence risk assessment and be applied in treatment planning in attempt to reduce the racially disparate rates of mortality from prostate cancer. Through whole genome copy number alteration and methylation scans, the study will identify individual and integrated DNA-based biomarkers of biochemical recurrence in 200 AA men (100 with and 100 without biochemical recurrence). These biomarkers will then be validated in an independent set of 200 AA men. In the first year of funding, we have enumerated both discovery and validation samples; have obtained formalin fixed paraffin embedded blocks from 300 of these men; have completed pathology review of 70 of the discovery sample tumors; macrodissected and performed DNA extraction from 50 tumors, which will serve as a pilot sample of both the methylation and copy number platforms; and completed the running and quality control of two tumors on the copy number assay. We have also started a manuscript exploring the effectiveness of a commonly used clinicopathologic predictor of prostate cancer in AA men and whether that effectiveness depends on genetic African ancestry, which is significant as we proposed that our genomic biomarkers would add to this established predictor.

15. SUBJECT TERMS
prostate cancer; DNA; copy number alterations; methylation; biomarker; racial disparities; multi-omics

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1. INTRODUCTION:

Approximately ~33,000 men die each year from prostate cancer (CaP), in particular from disease recurrence. African American (AA) men have higher CaP mortality rates than age matched European American (EA) males. Risk of disease recurrence after primary treatment is difficult to predict with clinical variables and prostate specific antigen. Robust methods for risk stratification of prostate tumors are needed to enable men and their physicians to safely select between post-treatment surveillance and immediate adjuvant therapy. The purpose of our research is to use a mulit-omic approach to identify somatic copy number alterations and methylation markers in the primary tumors of AA men that can serve as a component of their recurrence risk assessment and be applied in treatment planning in attempt to reduce the racially disparate rates of mortality from CaP. Through whole genome copy number alteration and methylation scans, the study will identify individual and integrated DNA-based biomarkers of biochemical recurrence in 200 AA men (100 with and 100 without biochemical recurrence). These biomarkers will then be validated in an independent set of 200 AA men.

2. KEYWORDS:

prostate cancer; DNA; copy number alterations; methylation; biomarker; racial disparities; integrative.

3. ACCOMPLISHMENTS:

- **What were the major goals of the project?**

Major Task #1: Identify subjects and tissue specimens for biomarker discovery and validation.

Identify from the existing database at Henry Ford Health System (HFHS) lists of eligible prostatectomy patients as defined in the Research Strategy, confirm availability of banked FFPE prostate tissue with biorepositories. Target completion January 31st 2016; Completed January 1st 2016

Calculate CAPRA-S scores for all eligible subjects. Target completion January 31st 2016; Completed January 15th 2016

Perform incidence sampling to determine discovery and validation study samples. Target completion September 1st 2016; Discovery sample 35% completed

Tumor blocks will be pulled from archive, determination of the optimal block, and sections cut and tumor areas marked by histopathologist. Target completion September 1st 2017; Discovery sample 35% completed

Pathology review of cut cases and slides transferred to UCSF. Target completion January 1st 2018; Discovery sample 35% completed

Major Task #2: Tissue processing and DNA extraction for entire project.

Manual tumor tissue macrodissection. Target completion January 1st 2018; Discovery sample 35% completed

DNA extraction and quality assessment. Target completion February 28th 2018; Discovery sample 35% completed

Major Task #3: Perform genomic microarray experiments.

Carry out array comparative genomic hybridization (aCGH) on Aim 1 DNAs at UCSF. Target completion date September 1st 2017; Reagents quotes obtained. Reagents ordered. 1st two samples run and passed QC.

Quality control for aCGH and determination of copy number via CBS. Target completion date September 1st 2017; 1st 2 samples run and passed QC

Conduct methylation microarray experiments on Aim 1 DNAs. Target completion date September 1st 2017; Worked out an agreement with Illumina to provide methylation reagents for 1st 50 samples. Reagents ordered. Established agreement with a core at UCS for array processing.

Quality control methylation microarray data and preparation of an analysis dataset. Target completion date September 1st 2017; not yet started.

Major Task #4: Statistical analyses for GEMCaP and published methylation biomarkers. Target completion November 1st 2017; not yet started.

Major Task #5: Discovery of African American specific copy number and methylation biomarkers. Target completion Febuary 1st 2018; not yet started.

Major Task #6: Validate integrated biomarker panel in a separate discovery set of African American prostate cancer. Target completion July 1st 2018; not yet started.

Major Task #7: Draft manuscripts for publication.

Manuscript #1: CAPRA-S performance in an African American population. Target completion March 2017; 50% completed.

What was accomplished under these goals?

In the current reporting period, our major activities were the identification of the study subjects, abstraction of their clinical and pathologic data, acquisition of their formalin fixed paraffin embedded (FFPE) tumor tissue blocks, pathologic review of each case, sectioning of blocks, and DNA extraction. To date, we have identified all 200 subjects for the discovery cohort, and their tissue blocks have been acquired. Of those, 51 (25.5%) have undergone pathologic review, have been sectioned, and have undergone DNA extraction. For the 200 validation cohort subjects, we have identified 159 (79.5%) of the subjects. Of those 159, we have currently retrieved FFPE blocks for 79 of the subjects. Following completion of the pathologic review of the subjects for the genome-wide discovery analyses, we will proceed with the pathologic review, sectioning, and DNA extraction for the validation study subjects.

As one of our objectives is to develop somatic DNA-based biomarkers that augment the ability of current clincopathologic tools (e.g. CAPRA-S) to predict recurrent disease and that the performance of these clinicopathologic predictors in AA men have not been well described in the literature, we performed an analysis comparing the effectiveness of the Cancer of the Prostate Risk Assessment Post-Surgical (CAPRA-S) score at predicting biochemical recurrence in AA and EA men. Our findings suggested that CAPRA-S performed similarly in both self-identified race-ethnicities. As AA are an admixed population, with ancestry derived from both Africa and Europe, we also asked the question if the effectiveness of CAPRA-S differed by the percentage of African ancestry in AA men. Our findings suggest no differences in the predictive ability of CAPRA-S based on percent genome-wide African ancestry. We presented these findings at the CDMRP 2016 Innovative Minds in Prostate Cancer Today meeting. Our results show that it's valid to use CAPRA-S in our analytic strategy to identify genomic alterations that add to this clinicopathologic predictor of BCR, leading to further improvements in clinical recurrence risk prediction in AA men and more refined identification of those likely to benefit from earlier adjuvant therapy. We are currently expanding the sample size for this analysis and plan to publish these findings in the next reporting period.

- **What opportunities for training and professional development has the project provided?**

  Nothing to report

- **How were the results disseminated to communities of interest?**

  Nothing to report

- **What do you plan to do during the next reporting period to accomplish the goals?**

  1. Complete the pathologic review of all 400 tumors; sections for all tumors will be cut and sent to UCSF for macrodissection, DNA extraction, and copy number array profiling.
  2. Complete manuscript on the performance of CAPRA-S in African American men and whether its effectiveness differs by genome-wide percent African ancestry.
  3. Complete a pilot study of 48 tumors (24 with and 24 without biochemical recurrence that are part of the discovery sample) to determine the sensitivity and specificity of the new Illumina EPIC methylation arrays to recover copy number alterations in the

tumors. All 48 tumors will be profiled using the methylation and copy number arrays, and the results from this pilot will also lead to a manuscript in the coming year.

4. Conduct all array experiments for the discovery sample.

4. IMPACT:

a. What was the impact on the development of the principal discipline(s) of the project?

Our findings of similar effectiveness of CAPRA-S in AA men in comparison to EA men is something that is not established in the literature. These results impact clinical care of AA men with prostate cancer as they establish that CAPRA-S can be used effectively in assessing risk of recurrence in this minority population that suffers disproportionately from prostate cancer.

b. What was the impact on other disciplines?

Nothing to report

What was the impact on technology transfer?

Nothing to report

c. What was the impact on society beyond science and technology?

Nothing to report

5. CHANGES/PROBLEMS:

a. Changes in approach and reasons for change

We have made the decision to not match our biochemical recurrence and non-recurrence subjects on CAPRA-S score as part of the design. Rather, we will adjust for CAPRA-S as part of our analysis to ensure that our biomarker(s) provide added value to CAPRA-S.

b. Actual or anticipated problems or delays and actions or plans to resolve them

The one delay for the reporting period is FFPE block retrieval and pathologic review. We have worked closely with our colleagues in pathology to solve both issues. The delay in the FFPE

block retrieval has been dealt with, and to date, nearly 75% of the 400 blocks (200 for discovery and 200 for validation) have been retrieved. Regarding pathologic review, we have worked closely with our pathologist (Dr. Nilesh Gupta) over the past eight months to streamline the process to maximize his review time. With the new process in place, we believe that we will be able to realistically complete the review of all 400 tumors by the end of the second year of the grant. We are closely monitoring the progress of the review. If the numbers from the first three months of the second year suggest that completion of the review by the end of year two is not realistic, we will enlist the aid of a second pathologist to complete the review.

c. **Changes that had a significant impact on expenditures**

Nothing to report

d. **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**

e. **Significant changes in use or care of human subjects**

Nothing to report

f. **Significant changes in use or care of vertebrate animals.**

Nothing to report

g. **Significant changes in use of biohazards and/or select agents**

Nothing to report

6. PRODUCTS:

a. **Publications, conference papers, and presentations**

Presentation #1 DoD IMPaCT Meeting 2016, Bethesda, MD: “The impact of self-identified race-ethnicity and genetic ancestry on a commonly used clinicopathologic predictor of biochemically recurrent prostate cancer”.

i. Journal publications.

Nothing to report

ii. **other non-periodical, one-time publications.**

Nothing to report

iii. **Other publications, conference papers, and presentations.**

Nothing to report

b. **Website(s) or other Internet site(s)**

Nothing to report

c. **Technologies or techniques**

Nothing to report

d. **Inventions, patent applications, and/or licenses**

Nothing to report

e. **Other Products**

Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

a. **What individuals have worked on the project?**

Name:	*Albert M. Levin, PhD*
Project Role:	*co-PI*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*3*
Contribution to Project:	*Dr. Levin is the PI for the Henry Ford site. In addition to the design of the study, he is overseeing the process of tissue acquisition, pathology review,*

	clinical/pathological data abstraction, histological staining and sectioning of the blocks, specimen shipment, data analysis, and manuscript writing.
Funding Support:	*DoD; The Fund for Henry Ford*

Name:	*Pamela L. Paris, PhD*
Project Role:	*co-PI*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*3*
Contribution to Project:	*Dr. Paris is the PI for the UCSF site, which is doing all of the DNA extractions and copy number array profiling. She is also working closely with Dr. Levin on oversight of pathologic review and tissue preparation, as well as development and writing of manuscripts based on the cohort.*
Funding Support:	*DoD*

Name:	*Benjamin A. Rybicki, PhD*
Project Role:	*co-I*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*1*
Contribution to Project:	*Dr. Rybicki has provided mentorship and guidance for all aspects of the study development for Dr. Levin. He also participates in the development and the writing of manuscripts for the study.*

Funding Support:	*DoD*

Name:	*Nilesh Gupta, MD*
Project Role:	*Pathologist*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*1*
Contribution to Project:	*Dr. Gupta is responsible for the pathologic review of all of the tumors from the cohort subjects.*
Funding Support:	*DoD; The Fund for Henry Ford*

Name:	*Sudha Sadasivan, PhD, MPH*
Project Role:	*Study coordinator*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*6*
Contribution to Project:	*Dr. Sadasivan is responsible for the day-to-day management of all aspects of the project.*
Funding Support:	*DoD; The Fund for Henry Ford*

Name:	*Khanh Kieu, BA*
Project Role:	*Laboratory technician*
Researcher Identifier (e.g. ORCID ID):	

Nearest person month worked:	*5*
Contribution to Project:	*Mr. Kieu is responsible for the acquisition of the tumor blocks, abstraction from the original pathologic review to determine within which blocks the index nodule is located, and normal tissue macrodissection, and normal tissue DNA extraction.*
Funding Support:	*The Fund for Henry Ford*

b. **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

Nothing to report

c. **What other organizations were involved as partners?**

Nothing to report

8. SPECIAL REPORTING REQUIREMENTS

Not applicable

9. APPENDICES:

Nothing to report